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Glucose transporter (GLUT1) allele (XbaI–) associated with nephropathy in non-insulin-dependent diabetes mellitus

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Glucose transporter (GLUT1) allele (XbaI–) associated with nephropathy in non-insulin-dependent diabetes mellitus.

Background. Although multiple factors contribute to the initiation and progression of diabetic nephropathy (DN), hyperglycemia and genetic predisposition are two major components implicated in the development of DN. Several pieces of experimental evidence suggest that glucose transporter (GLUT1) activity is an important modulator for the cell hypertrophy and extracellular matrix formation of glomerular mesangial cells.

Methods. To evaluate the role of the GLUT1 gene mutation in the development of DN in Chinese patients with non-insulin-dependent diabetes mellitus (NIDDM), the polymorphic XbaI site of GLUT1 gene was analyzed by polymerase chain reaction in 124 normal subjects and 131 patients with NIDDM, among whom 64 were complicated with DN. DN was defined as persistent albuminuria with or without impaired renal function with no known cause of renal disease other than diabetes.

Results. The frequencies of XbaI (+/−) genotype (75 vs. 44%, $P < 0.01$) and XbaI (−) allele (44 vs. 29%, $P < 0.05$) were significantly higher in NIDDM patients with DN than those without nephropathy. There were no significant differences for GLUT1 genotype and allele frequency between NIDDM patients without nephropathy and normal subjects. The presence of the XbaI (−) allele appeared to have a strong association with the development of DN. The odds ratio was 1.915, and the 95% confidence interval was 1.044 to 3.514. In addition, no strong association was found between GLUT1 gene polymorphism and retinopathy in NIDDM patients.

Conclusion. Our results indicate that the XbaI (−) allele of the GLUT1 gene might be a genetic marker of NIDDM with DN, and this genetic susceptibility is independent of its retinopathy in Chinese subjects.

Nephropathy is a major cause of morbidity and mortality in diabetes mellitus. Non-insulin-dependent diabetes mellitus (NIDDM) accounts for approximately 5% of end-stage renal diseases in China, and the frequency is increasing rapidly in the recent years [1]. NIDDM is a metabolic disorder characterized by chronic hyperglycemia caused by impaired glucose metabolism. Nowadays, most diabetic patients eventually experience one or more of the long-term complications of this chronic disease. Diabetic retinopathy, nephropathy, and cardiovascular disorders are the most conspicuous complications. However, unlike retinopathy, which eventually affects a majority of the diabetic patients over time, nephropathy developed in only a limited population of approximately 30 to 40% of the whole group [2, 3]. Such an epidemiological phenomenon suggests that a subset of diabetic individuals are particularly susceptible to diabetic nephropathy (DN). Strong familial aggregation of the disease suggests that genetic components play a major role as determinants of susceptibility to the disease. The inherited defects in the greater part of the common form of NIDDM, however, remain to be identified [3, 4]. Diabetes results from the combined deficiencies of insulin secretion, insulin action, glucose uptake, and utilization. Efforts have been made to search for the gene polymorphism of these physiological actions. However, even taking advantage of the recent progress in biotechnology, population-association studies of several candidate genes for the etiology of NIDDM, including the glucose transporter genes glucose transporter-1 (GLUT1) and GLUT2 [5, 6], the insulin receptor gene [7], the insulin gene [8], the insulin receptor substrate-1 gene [9, 10], and the glucokinase gene [11], have thus far yielded inconclusive results.

Glucose transporters are membrane-embedded proteins that mediate the uptake of glucose from the surrounding medium into the cells. Glucose is the main fuel for most cells, and its uptake is rate limiting for a more efficient utilization [12, 13]. Immunogold labeling and *in situ* hybridization studies showed that glucose transporters are widely distributed from glomeruli down to the inner medullary collecting duct in normal kidney [14, 15]. It has been demonstrated that GLUT1 is the main transporter of glucose in the glomeruli [15, 16]. As alterations of glomerular mesangial cells constitute the most

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common pathological changes of DN and contribute to the kidney failure in this disease, we hypothesized that GLUT1 gene mutation might contribute to the development of DN. To evaluate the role of mutation of the GLUT1 gene in the development of renal damage in NIDDM, a gene population study was conducted in a group of Chinese NIDDM patients with or without nephropathy and normal subjects using the XbaI polymorphism of the GLUT1 gene.

METHODS

Patients and controls

Glucose transporter-1 gene polymorphism was assessed in 131 patients of NIDDM (83 males and 48 females, ranging in age from 46 to 71) with or without nephropathy and 124 nondiabetic control subjects (58 males and 66 females, ranging in age from 36 to 54). All patients enrolled in this study were diagnosed with NIDDM according to the criteria based on the World Health Organization report of 1985. They were recruited from the patients attending the nephrological clinic, being hospitalized for a thorough examination. Twenty-four hour urinary albumin excretion (UAE) was measured for each diabetic patient on three separate occasions during the observation period. Urinary albumin concentration was measured within 10 days of storage at the standard refrigerator temperature. UAE was assayed by radioimmunoassay. For this study, the definition of overt albuminuria was an UAE measurement of 200 μg/min or more. DN was defined as the presence of persistent albuminuria or proteinuria with or without impaired renal function, with no known cause of renal disease other than diabetes. According to this definition, 64 cases diagnosed as having the complication of DN (40 males and 24 females, ranging in age from 48 to 67), among whom 40 patients underwent renal biopsy, all showed the histological changes of DN. Cases showing evidence of parenchymatous renal disease other than the DN or urinary tract diseases were excluded from the study. Eighty-five out of the 120 cases with NIDDM in this group were screened for diabetic retinopathy. Body mass index (BMI) of all of the cases was calculated as weight/height^2 (kg/m^2). All of the cases and normal control subjects included in this study were of Han nationality.

Genomic DNA preparation

DNA was extracted by standard methods from peripheral blood monocytes. Genomic DNA suspended in 10 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0, and the concentration was measured by spectrophotometry.

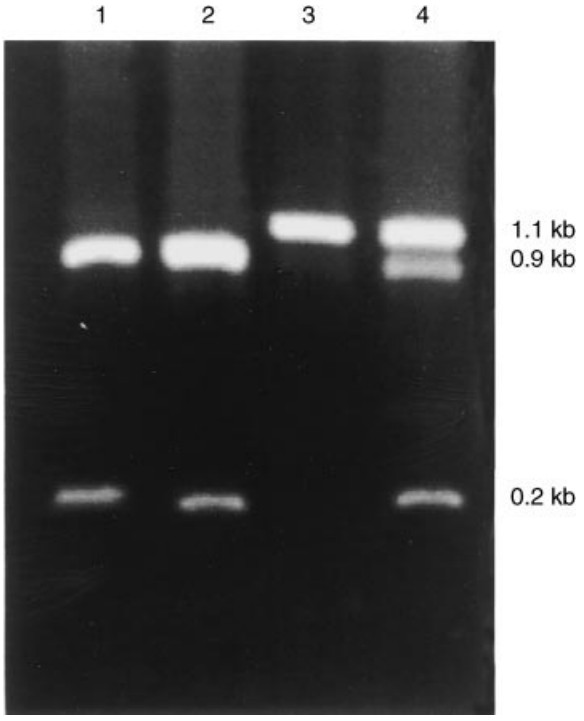

Fig. 1. XbaI polymorphism of human GLUT1 gene detected by PCR-RFLP. Lanes 1 and 2 are the XbaI (+/+) genotype. Lane 3 is the XbaI (−/−) genotype. Lane 4 is the XbaI (+/−) genotype.

XbaI restriction fragment length polymorphism (RFLP) of the human GLUT1 gene

The polymorphic XbaI site is localized in the second intron of the gene by restriction enzyme analysis of a human GLUT1 genomic clone. In the mutant form, guanine (G) has been transversed to thymine (T) and abolished the recognition site. A 5′ primer (TGT GCA ACC CAT GAG CTA A) and a 3′ primer (CCT GGT CTC ATC TGG ATT CT) were synthesized. A 1.1 kb DNA fragment including the polymorphic XbaI site was amplified by polymerase chain reaction (PCR) [6]. After 30 cycles of amplification consisting of denaturation at 94°C for one minute, annealing at 55°C for one minute, extension at 72°C for 1.5 minutes, the PCR products were digested with XbaI restriction enzyme and electrophoresed on a 1.2% agarose gel. XbaI RFLP was detected by ethidium bromide staining. A 1.1 kb band corresponded to the XbaI (−) allele, and a set of 0.9 and 0.2 kb bands corresponded to the XbaI (+) allele (Fig. 1).

Statistics

Allele frequency means the number of occurrences of the test allele in the population divided by the total number of alleles. Results obtained from the control and test groups were compared using the χ^2 test to show statistical significance. In each group, the observed distribution of homozygotes and heterozygotes group con-

Table 1. Genotype distribution and allele frequency of the GLUT1 gene in NIDDM with or without nephropathy

		Genotype			Allele frequency	
	N	XbaI(+/+)	XbaI(−/+)	XbaI(−/−)	XbaI(+)	XbaI(−)
NIDDM	131	43(0.33)	78(0.59)[d]	10(0.08)	164(0.63)	98(0.37)[d]
DN(+)	64	12(0.19)	48(0.75)[ab]	4(0.06)	72(0.56)	56(0.44)[ac]
DN(−)	45	22(0.49)	20(0.44)	3(0.07)	64(0.71)	26(0.29)
Controls	124	77(0.62)	41(0.33)	6(0.05)	195(0.79)	53(0.21)

[a] $P < 0.001$ vs Control
[b] $P < 0.01$ vs DN(−)
[c] $P < 0.05$ vs DN(−)
[d] $P < 0.01$ vs Control

Table 2. Baseline characteristics of the NIDDM patients according to GLUT1

	Genotype		
	XbaI(+/+) $N = 43$	XbaI(+/−) $N = 78$	XbaI(−/−) $N = 10$
Gender male/female	17/26	27/51	4/8
Age *years*	60.7 ± 12.0	58.4 ± 13.3	59.5 ± 12.4
History of diabetes *years*	9.8 ± 7.6	8.5 ± 7.0	9.9 ± 8.5
Systolic BP *mm Hg*	145.8 ± 4.0	142.5 ± 3.5	146.8 ± 3.0
Diastolic BP *mm Hg*	85.6 ± 2.0	83.4 ± 1.4	85.9 ± 1.5
Glycosolated hemoglobin %	7.5 ± 1.2	7.3 ± 1.1	7.4 ± 1.4
BMI kg/m^2	26.2 ± 3.6	25.4 ± 6.6	24.9 ± 5.0

formed to expectation based on the Hardy–Weinberg equilibrium analysis. Odds ratios and their corresponding confidence intervals were then calculated from the logistic regression parameter estimates. A P of less than 0.05 was regarded as statistically significant.

RESULTS

The distribution of the GLUT1 gene polymorphism in 131 NIDDM patients and those with or without nephropathy is shown in Table 1. Twenty-two out of 131 NIDDM patients who did not have the qualified record of a 24-hour UAE were excluded from the study of comparison between diabetics with or without nephropathy. The frequency of XbaI (+/−) genotype in DN was much higher (75 vs. 44%, $P < 0.01$), whereas the XbaI (+/+) genotype was apparently lower (19 vs. 49%, $P < 0.01$) than the group of NIDDM patients without nephropathy. The allele frequency of XbaI (−) was also significantly higher in the former group than the latter one (44 vs. 29%, $P < 0.05$). It is important to point out that the distribution of GLUT1 genotype frequency for the NIDDM patients without nephropathy showed no substantial difference to that of the normal controls. Therefore, the presence of the XbaI (−) allele virtually had a strong association with the development of DN instead of the NIDDM *per se.* The odds ratio was 1.915, and the 95% confidence interval was 1.044 to 3.514.

To assess the relationship of GLUT1 genotype and the baseline characteristics of the NIDDM patients, 131 cases of NIDDM were categorized according to their genotype. Baseline characteristics were compared over the three genotypes (Table 2). Table 2 shows that these baseline characteristics were similar for the three different genotypes.

To examine the influence of GLUT1 gene polymorphism on the development of retinopathy complication and its correlation with DN in NIDDM patients, 45 patients with proliferative retinopathy were divided into two groups according to whether they were complicated with DN (Table 3). The genotype distribution of GLUT1 showed that there was a significant difference between the group of retinopathy patients with DN and the normal subjects. However, there was no difference between the retinopathy patients without DN and the normal subjects.

DISCUSSION

Among all of the long-term complications of diabetes, nephropathy imposes the highest social and economic burden. DN, which progresses inexorably toward renal failure, is a common indication for hemodialysis and renal transplantation. In addition, DN and its related metabolic alterations accelerate the progression of atherosclerotic lesions, further increasing the already existing high risk of coronary artery disease. Although multiple factors contribute to the initiation and progression of DN, hyperglycemia and genetic predisposition are the two major components implicated in the development

Table 3. Relationship between the GLUT1 genotype and diabetic retinopathy in NIDDM patients

		Genotype			Allele frequency	
	N	XbaI(+/+)	XbaI(+/−)	XbaI(−/−)	XbaI(+)	XbaI(−)
Retinopathy DN(+)	37	10(0.27)[b]	22(0.59)[b]	5(0.14)[a]	42(0.56)[b]	32(0.43)[b]
Retinopathy DN(−)	8	3(0.37)	4(0.50)	1(0.13)	10(0.62)	6(0.38)
Controls	124	77(0.62)	41(0.33)	6(0.05)	195(0.79)	53(0.21)

[a] $P < 0.05$ vs. Controls
[b] $P < 0.01$ vs. Controls

of DN [17]. It has been noted that only a limited number of diabetic patients experience DN. The prevalence of DN is considered to be approximately 40% in both types of diabetes, but there is a wide variation among different ethnic groups [17]. A long-term study of diabetic patients who received kidney transplants because of the development of end-stage renal disease caused by DN showed that only approximately 50% of the patients had a recurrence of nephropathy during a post-transplantation period of 6 to 14 years. This suggested that there might be a difference in the donor kidney's resistance to the development of DN [18]. In diabetes, an obvious injurious impact on the host cells is the continued presence of an abnormally high concentration of extracellular glucose. Nonetheless, there is also evidence suggesting that the mere presence of high blood glucose level is not sufficient to cause the development of DN [19]. Observations of familial clustering of renal complications and the fact that the incidence of DN varied in different racial groups suggest that genetic background may be the most important determinant in the causation of diabetic renal damage. It is therefore hypothesized that the investigation of genetic susceptibility to DN will shed light on the pathogenesis of renal involvement in diabetes.

The renal glomerular lesions of human and experimental diabetes mellitus are characterized by glomerular hypertrophy and the deposition of extracellular matrix in the form of diffuse thickening of the glomerular basement membrane and mesangial expansion [20, 21]. *In vitro* study proved that the mesangial cells increased their production of extracellular matrix when incubated in medium with a high concentration of glucose [22, 23]. It is conceivable that the metabolic derangement of renal cells, particularly mesangium, resulting in the excessive formation and deposition of extracellular matrix component in diabetes is a likely determinant of mesangial expansion and glomerulosclerosis. Accordingly, the regulation of glucose transporter-mediated mesangial glucose uptake might be crucial in order to understand the pathophysiology of DN. GLUT1 is the main glucose transporter in the glomeruli. Previous studies demonstrated that rat mesangial cell expressed GLUT1 and GLUT4 [24, 25]. Recently, to our knowledge for the first time, our *in vitro* work proved that human mesangial cells also express functional GLUT1 in culture (abstract; Liu et al, *J Am Soc Nephrol* 9:636A, 1998). Interestingly, it has been reported that cultured rat mesangial cells transduced with the human GLUT1 gene demonstrated a markedly increased glucose uptake and metabolism as well as significant stimulation of extracellular matrix synthesis, even when the cells were grown in a normal extracellular glucose concentration [26]. This work suggests that increased glucose uptake, rather than the extracellular concentration of glucose *per se*, would be a major metabolic determinant in the development of mesangial expansion and glomerulosclerosis in diabetics. It is plausible that the expression and activity of mesangial GLUT1 in diabetic patients varies according to their genetic background, and this variation could explain why only a limited group of diabetic patients are predisposed to the development of renal disease, and could also clarify the reason for the poor correlation between glycemic levels and progression of nephropathy in a subset of diabetics. Based on these assumptions, GLUT1 was chosen as a candidate gene for the study of polymorphism in our investigation.

The evidence shows that the XbaI (+/−) genotype and XbaI (–) allele frequency were significantly higher in NIDDM patients with DN as compared with either normal subjects or NIDDM patients without DN. The absence of an association between the XbaI (−/−) genotype and DN probably is due to the relatively low frequency of XbaI (−/−) genotype and the small number of investigated subjects. Our investigation suggests that the GLUT1 polymorphism may be an important genetic marker for the development of DN in NIDDM of Chinese patients. NIDDM patients with the XbaI (−) allele are more likely to develop DN as compared with patients without the XbaI (−) allele. Previous studies from the literature on the relationship of GLUT1 gene polymorphism and NIDDM yield contradictory results. Elbein et al, who examined the linkage of the GLUT1 genotype with NIDDM in 18 large Utah white pedigrees, failed to find a genetic predisposition [5]. On the other hand, Tao et al, in an analysis of 91 Japanese NIDDM patients and 87 nondiabetic control subjects, found that the XbaI (−) allele of the GLUT1 gene appeared to be a genetic marker of NIDDM in their subjects [6]. Of course, this

controversy might be due to racial differences. Results obtained from the Japanese may be different from those coming from the Caucasians. However, another reasonable explanation for this contradiction may be because these investigations did not pay special attention to the renal involvement of the NIDDM cases in their study populations. As shown in our data (Table 1), there was also a close association between XbaI (−) allele of GLUT1 and 131 NIDDM cases in general, but when we further dissected the result by dividing the investigated cases into two categories according to their absence or presence of renal complication, it was fascinating to find out that there were actually no significant differences between those NIDDM patients without DN and the normal subjects, as far as their genotype or allele frequency of the GLUT1 gene was concerned. The positive association of XbaI (−) allele for the NIDDM cases in general is due to a skewed population, which includes a large number of DN patients who happened to be in a clinic mainly serving the renal patients. On the other hand, the presence of the XbaI (−) allele also in part of the diabetics without nephropathy points to the possibility that the XbaI (−) allele may be only one of the multiple risk factors for the development of DN. It is imaginable that the pathogenesis of a complex renal complication such as DN has multiple inducing factors. It is also possible that the XbaI (−) allele does not function simply as a diabetes-independent risk factor for nephropathy.

Generally, the complications of diabetic mellitus manifest in a constellation. However, the annual and cumulative incidences of DN, diabetic retinopathy, and neuropathy are different [27]. For instance, it has been reported that among diabetics developing diabetic retinopathy, 61% already had diabetic neuropathy, but only 24% had DN [28]. These data suggest that the occurrence of ophthalmic and renal complications of diabetes may be influenced by organ-specific pathogenetic factors in addition to their common pathogenesis. The results of our study with regards to the GLUT1 gene polymorphism in diabetic retinopathy support this concept. The distribution of GLUT1 gene polymorphism in these cases did not show a strong association with retinopathy. It is also evidence indicating that the close association of XbaI (−) allele in NIDDM is mainly a gene marker for DN patients.

Our investigation demonstrates that NIDDM patients with the XbaI (−) allele of the GLUT1 gene may be prone to develop DN in Chinese subjects. This genetic susceptibility presumably functioning as a risk factor in conjunction with other factors of diabetes yields a higher incidence of nephropathy. Is this gene polymorphism of GLUT1 also able to influence the progression of DN and its clinical course? Our study is unable to answer these questions, as a large sample population and both prospective and long-term investigations are needed to provide definitive answers. Of course, there are multiple genetic factors influencing the development and outcome of diabetic nephropathy. GLUT1 gene polymorphism may be one of them that is found to be meaningful in its pathogenesis.

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